

What is claimed is:

1. A method of amplifying an amplification sequence of a target nucleic acid sequence comprising:

(a) contacting said amplification sequence with a plurality of pairs of amplification probes, wherein the member probes of each of said pairs of amplification probes are complementary to each other and at least one same hybridizing member of each pair of probes is also complementary to a portion of said amplification sequence. ←;

(b) allowing said hybridizing members of said amplification probes to hybridize to a different portion of said amplification sequence, with said amplification probes binding to said amplification sequence in a contiguous manner;

(c) causing said hybridized amplification probes to join together to form an amplification product;

(d) effecting separation of said amplification product from said target sequence; and,

(e) repeating steps (a) through (d).

2. The method of claim 1 wherein said hybridized amplification probes are joined together by the action of an enzyme.

3. The method of claim 2 wherein said enzyme is a ligase.

*all of plural
are pairs
hybridized? if so,
may not hybridize
each is
complementary
if some sequences
as target
self-complementary
must complement
can be
incomplete
hybridize*

Scope →

*This gives only
many complementary
copies of amp. seq.!*

*Enzyme
isolated
from E. coli*

4. The method of claim 1 wherein said hybridized amplification probes are joined together through a chemical reaction.

5. The method of claim 1 wherein at least three pairs of amplification probes are used.

6. A method for detecting a nucleic acid sequence having three or more ligated nucleic acid segments comprising:

(a) contacting said nucleic acid sequence with two detection probes, wherein each of said detection probes is complementary to a portion of each of two of said ligated nucleic acid segments which are adjacently situated in said nucleic acid sequence;

(b) allowing each of said detection probes to hybridize to two adjacently situated segments of said nucleic acid sequence, with said detection probes binding to said nucleic acid sequence sufficiently adjacent to each other to enable an interaction to occur between said hybridized detection probes;

(c) detecting the presence of said hybridized detection probes.

7. The method of claim 6 wherein at least one of said detection probes is labeled.

8. The method of claim 7 wherein one of said detection probes is labeled with a first proximity label and the other of said detection probes is labeled with a second proximity label.

9. The method of claim 6 further comprising:

(a) causing said hybridized detection probes
5 to join together to form a ligated detection product;
and,

(b) detecting the presence of said ligated
detection product.

10 The method of claim 9 wherein said
hybridized detection probes are joined together by the
action of a enzyme. *ligase*

11. The method of claim 10 wherein said
15 enzyme is a ligase.

12. The method of claim 9 wherein said
hybridized detection probes are joined together through
a chemical reaction.

20 13. The method of claim 9 wherein one of said
detection probes is labeled with a detectable label and
the other of said detection probes is labeled with a
means for removing said ligated detection product from
25 solution.

✓ 14. A method for detecting a target nucleic
acid sequence which may be present in a test sample
comprising:

30 (a) contacting said test sample with a
plurality of pairs of nucleic acid amplification probes,
wherein the member probes of each of said pairs of
amplification probes are complementary to each other and
35 at least one same hybridizing member of each pair of
probes is also complementary to an amplification
sequence of said target nucleic acid sequence;

Sub Q3
have detect?
see cl 6

not ligated
enzyme

7.33
Use
in duplicate
and not
embodiment
(labeled w/
any antibody?)

see cl 1
(expt 4)
Sub Q4

how can make
show one member
hybridize to 2nd
amp. seq. of (a)

(b) allowing said hybridizing members of said
amplification probes to hybridize to a different portion
of said amplification sequence, with said amplification
probes binding to said amplification sequence in a
5 contiguous manner;

(c) causing said hybridized amplification
probes to join together to form an amplification
product;

10
Sub
a4

(d) effecting separation of said amplification
product from said amplification sequence;

(e) contacting said amplification product with
15 two detection probes, wherein each of said detection
probes is complementary to a portion of each of two of
said amplification probe segments which are adjacently
situated in said amplification product;

see cl 6
(ex- 4 3...)
no antiseedol base
for amp. probe segment

20 (f) allowing each of said detection probes to
hybridize to two adjacently situated segments of said
amplification product, with said detection probes
binding to said amplification product sufficiently
adjacent to each other to enable an interaction to occur
25 between said hybridized detection probes;

only have 1
hybridized probe
duplex

(g) detecting the presence of said hybridized
detection probes.

30 15. The method of claim 14 further
comprising:

(a) causing said hybridized detection probes to
join together to form a ligated detection product; and,

35
see cl 9

(b) detecting the presence of said ligated detection product.

16. The method of claim 14 wherein said
5 hybridized amplification probes are joined together by
the action of an enzyme.

17. The method of claim 15 wherein said
enzyme is a ligase.

10

18. The method of claim 14 wherein said
hybridized amplification probes are joined together
through a chemical reaction.

15

19. A reagent for use in the amplification of
an amplification sequence comprising a plurality of
pairs of nucleic acid amplification probes, wherein the
member probes of each pair of amplification probes are
complementary to each other and at least one same
hybridizing member of each pair of amplification probes
is also complementary to a given portion of said
amplification sequence, with the nucleic acid sequences
of each pair of amplification probes selected to be
complementary to a different portion said amplification
sequence, the amplification probes being capable of
hybridizing to the amplification sequence in a
contiguous manner sufficiently adjacent to each other to
enable the probes to be joined together.

25

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20. A reagent for use in the detection of a
nucleic acid sequence having three or more ligated
nucleic acid segments comprising two nucleic acid
detection probes, wherein each of said detection probes
is complementary to a portion of each of two of said
ligated nucleic acid segments which are adjacently
situated in said nucleic acid sequence, with at least

35

ligase

amp. seq. of env.?

see cl 1
(except concd buffer?)
("inc hybrid")

Jul
as

ref to 3
or more, or NA seq?

see cl 6
(5 have detect?)

one of said detection probes being provided with a label, the detection probes being capable of hybridizing to said nucleic acid sequence sufficiently adjacent to each other to enable an interaction to occur between
5 said detection probes.

21. A kit for use in the detection of a target nucleic acid sequence which may be present in a test sample comprising:

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(a) a plurality of pairs of amplification probes, wherein the member probes of each pair of amplification probes are complementary to each other and at least one same hybridizing member of each pair of amplification probes is also complementary to an amplification sequence of said target nucleic acid sequence, with the nucleic acid sequences of each pair of amplification probes selected to be complementary to a different portion of said amplification sequence, said
20 amplification probes being capable of hybridizing to said amplification sequence in a contiguous manner sufficiently adjacent to each other to enable the probes to be joined together to form an amplification product; and,

25

(b) two detection probes, wherein each of said detection probes is complementary to a portion of each of two amplification probe segments of said amplification product which are adjacently situated in said amplification product, with at least one of said detection probes
30 being provided with a label, said detection probes being capable of hybridizing to said amplification product sufficiently adjacent to each other to enable an interaction to occur between said hybridized detection probes; ←.

35

add
B7

see cl 19
(except ampl seq)
Sub
A5

see cl 79
(except seq has...)